INCORPORATING ELECTROSPUN FIBERS INTO ULTRA-STRONG HYDROGELS

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

Incorporating Electrospun Fibers into Ultra-Strong Hydrogels

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Hydrogels are hydrophilic polymer networks that exhibit high biocompatibility due to their high water content. In applications such as musculoskeletal repair, their main limiting factor is their relatively low mechanical strength. By increasing mechanical strength and toughness, the application range of these hydrogels can be widened. In previous work, ultra-strong, double network (DN) hydrogels have been developed which exhibit improved mechanical properties, with a compressive strength ~ 25.8 MPa and a compressive modulus ~ 1.2 MPa. However, these DN hydrogels showed poor resistance to injury, limiting their clinical applications.

In this work, electrospun PCL fibers were incorporated into the ultra-strong DN hydrogels as a physical reinforcement to further improve their mechanical properties. Notably, a mesh plate collector was utilized to form the PCL fibers into a woven pattern via electrospinning, creating a highly organized fiber mesh. By integrating the PCL fiber meshes into the ultra-strong DN hydrogels, the high tensile strength (~1.2 MPa) was maintained while the tensile modulus (~1.8 MPa) and tear resistance (>4x DN hydrogel without fibers) were significantly enhanced. Furthermore, DN mesh-gels were fabricated with exposed fibers to permit direct suturing to tissues or preexisting devices, previously not achievable with most hydrogel materials.

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CHAPTER I

INTRODUCTION

Background Information

In the case of tissue or organ failure, a full or partial transplant requires an acceptable healthy substitute, whether as an autograft or allograft. In the case of knee articular cartilage, damaged cartilage is replaced by cartilage harvested from another part of the knee in autografting knee surgery.[1] This process causes unnecessary injury during the procedure and can present complications such as donor site morbidity,[2] limited tissue availability,[3] and limitations on the possible geometry of the autograft due to the configuration of the donor site.[4] A tissue-engineered alternative would mitigate these limitations associated with autografting.

Hydrogels are hydrophilic polymer networks that exhibit excellent biocompatibility due to their high water content. They have been utilized in numerous applications in the medical field such as in contact lenses[5] and controlled drug delivery.[6] In applications such as musculoskeletal repair, their main limiting factor is their relatively low mechanical strength.[7,8,9] By increasing mechanical strength and toughness, the application range of these hydrogels can be widened.

In order to achieve this goal, Grunlan *et al.*[10,11] has reported ultra-strong, double network (DN) hydrogels, which are composed of a tightly crosslinked, 1st network hydrogel comprising of 2-acrylamido-2-methylpropane sulfonic acid (AMPS) with a loosely crosslinked, 2nd network of *N*-isopropylacrylamide (NIPAAm) copolymerized with acrylamide (AAm). These DN hydrogels, with two asymmetrically crosslinked, interpenetrating networks, exhibit significantly improved mechanical properties than their SN hydrogel counterparts of each network, with a compressive strength ~ 25.8 MPa and a compressive modulus ~ 1.2 MPa. However, their poor resistance to

injury remains a limitation for applications that require high tear resistance. Therefore, to improve this resistance to injury, a method of reinforcement will be utilized to form an ultra-strong and resilient composite DN hydrogel.

Reinforcement Approach

When attempting to improve the mechanical performance of any material, reinforcement is a practical option that is often employed. For instance, rebar (or reinforcement bar) is used to strengthen and hold tension in concrete.[12] The same principle is proposed here: incorporation of electrospun PCL fibers into ultra-strong hydrogels to further improve their mechanical properties. Electrospinning has been used as a method to synthesize material for almost 90 years, first patented in 1934.[13] The process involves a strong electric field generated between a capillary tip containing a polymer and a metallic collector. This charges the polymer inside the tip of the capillary, eventually overcoming the surface tension within and a jet of polymer whips out to form randomly aligned fibers on the collector. In this case, a plain, flat aluminum plate will be used as a collector, as well three different sized mesh plates. Using a mesh plate as a collector will organize the PCL fibers into a mesh pattern, facilitating formation of the ultra-strong hydrogel around the fiber mat. Other investigations[14] involving fibers to reinforce biomaterials tend to use scaffold materials that lack the hydration of hydrogels desired for many biomedical applications. In the few instances in which hydrogels are used,[15] they have not incorporated a double network structure. In this investigation, PCL fibers will be integrated into a unique, ultra-strong, double network hydrogel to act as reinforcement to improve mechanical properties such as modulus and tear resistance while maintaining the desirable properties of a hydrogel.

In addition to the improving mechanical properties, the embedded fibers could allow the gels to be sutured to a variety of soft tissues, resolving a common issue of graft loosening post-

implantation.[16,17] Furthermore, these composite hydrogels could be fabricated with exposed fibers that would serve as direct sutures to the surrounding tissue, allowing for easier, more stable attachment. These improvements could lead to a new generation of composite hydrogels that are able to withstand the mechanical requirements of implantation through their resistance to tearing and their capacity to be sutured directly and securely to adjacent tissues or other devices.

CHAPTER II

METHODS AND MATERIALS

Materials

N-isopropylacrylamide (NIPAAm) (97%), acrylamide (AAm), 2-acrylamido-2-methylpropane sulfonic acid (AMPS) (97%), polycaprolactone (MW 80k g mol^{-1}), anhydrous chloroform, *N*,*N*′- methylenebisacrylamide (BIS, 99%), 2-oxoglutaric acid (>99%) and ethanol were obtained from Sigma Aldrich.

Electrospinning PCL Fibers

To fabricate the fibers to reinforce the hydrogels in this investigation, electrospinning was used to form PCL fiber mats.[18] The solution utilized in this technique was made by mixing 9:1 anhydrous chloroform:ethanol and adding 15 wt% PCL (number average molecular weight, M_n~80k). This solution was pumped by a syringe pump through an 18G needle at a rate of 2 ml/hr. The 6 cm diameter collecting plate (or wire mesh [hole sizes: 0.10 mm, 1.30 mm, and 2.50 mm]) was placed 10 cm from the tip of the needle. A voltage source supplied 12.5 kV between the needle and collector.

In another part of the investigation, NIPAAM was incorporated into the PCL solution. The original fiber solution was adjusted so that NIPAAm replaced 5 wt% of PCL, making a solution comprised of 10 wt% PCL and 5 wt% NIPAAM. These fiber mats were only spun onto small-size meshes, due to the results found in the previous study.

Incorporating Fibers into Hydrogel Solution

After the mats were taken off of the collector, they were soaked in the 1st network solution, comprising of 1.5 M 2-acrylamido-2-methylpropane sulfonic acid (AMPS) as well as 4 mol%

crosslinker (N,N'-Methylenebisacrylamide) and 0.1 mol% photocatalyst (2-oxoglutaric acid). All bubbles were manually removed from the mat, and the solution/fiber complex cured between two glass slides under UV light for 5 hrs. The resulting 1st network hydrogels were then soaked in 2nd network solution comprising of 2 M *N*-isopropylacrylamide (NIPAAm) and 10 wt% acrylamide (AAm, wt with respect to NIPAAm), as well as 0.1 mol% crosslinker (N,N'-Methylenebisacrylamide) and 0.1 mol% photocatalyst (2-oxoglutaric acid). After a second cycle of the UV light curing process, the gels were soaked in DI water for at least 4 days until they were ready to be tested. Control (non-fiber containing) hydrogels were produced in similar fashion, without the addition of PCL fibers in the first step. This method was adapted from a previous publication from our group.[11]

Evaluating Gels and Meshes

In order to test whether adding PCL fibers to ultra-strong hydrogels improved their mechanical properties, a mechanical testing machine (Instron 3340) was utilized to procure the tensile modulus and strength. Hydrogels were punched into 3 dog-bone specimens (3 mm width, ~30 mm gauge length) with a die. Each sample was placed in the tensile clamps with an initial pre-load force of 0.2 N and then tested at a constant strain rate of 10 mm min^{-1} until fracture. The elastic compressive modulus (E) was obtained from the slope of the linear portion of the stress-strain curve from 0 to 10% strain. The ultimate tensile strength (σ_f) and the % strain at break (ε_f) was defined respectfully as the stress and strain values at the point of fracture. In addition, scanning electron microscopy (SEM) was used to evaluate fiber morphology prior to and after incorporation into the DN hydrogel.

Measuring Resistance to Injury

To measure the gel's ability to resist tearing, a notch test was performed.[19] In this test, an initial ~3 mm cut or "notch" was made in the gel slabs (~10 mm width, ~50 mm length) using a razor blade. Each sample was placed in the tensile clamps with an initial pre-load force of 0.2 N and then tested at a constant strain rate of 10 mm min^{-1} until fracture. This procedure is outlined in Figure 1. Images were taken during notch testing to qualitatively examine the tear mechanism of both fiber containing and plain hydrogel samples. To quantitatively evaluate the tear resistance, the percent strain the gel experienced at complete fracture (ε_f, when the gel was completely severed) was recorded. Toughness during the notch test was calculated by taking the area under the stress strain curve up to the point of complete fracture.

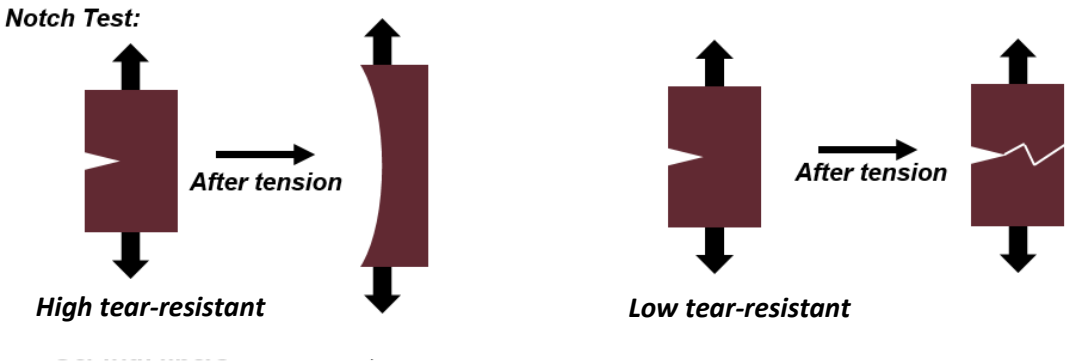

Figure 1. The process of notch testing. A high tear-resistant gel will withstand a higher stress before fracture after the introduction of the "notch," while a low tear-resistant gel will fracture after minimal stress is applied.

CHAPTER III

RESULTS

Combining Mesh and Gel

Different sized wire meshes were used to produce fiber mats organized around pores of varying sizes, ranging from approximately 0.10 mm to 2.50 mm. These were subsequently put into gels in the aforementioned manner.

One challenge that needed to be addressed with the fibers was getting the single network solution of the gel to integrate more effectively into the fiber network. When the meshes were incorporated into the first network solution, air bubbles would form, causing abnormalities in the finished mesh-gel (Figure 2), causing weakness and failure at these points. One method explored to address this challenge was to perform a plasma treatment on the fiber mats before exposing them to the solution; however, this method was determined to be ineffective. Another possible solution was to manually “press” the liquid into the mats, forcing out any air bubbles. This proved more effective, but still produced a few minor inconsistencies during fabrication.

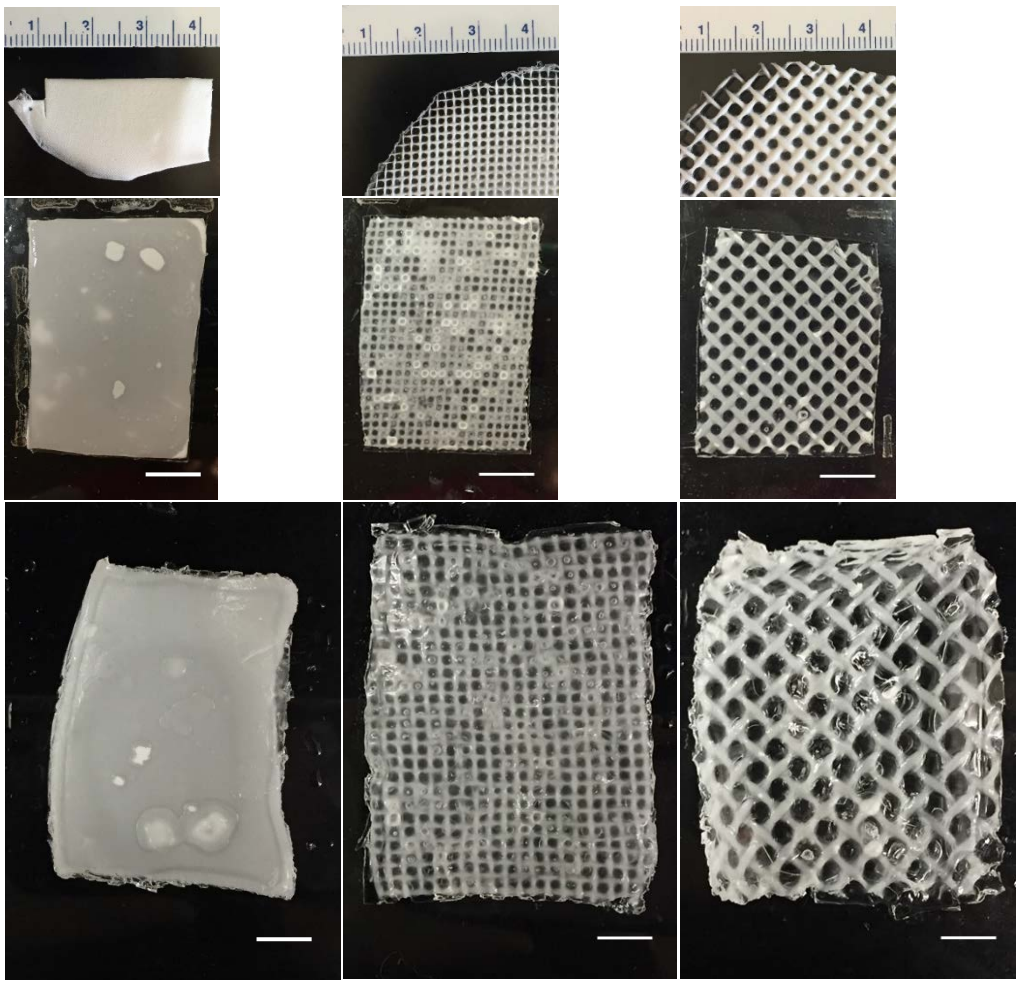

Figure 2. Plain PCL meshes before soaking in hydrogel solution (top row), mesh-gel after curing in first network hydrogel (second row), and mesh-gel after curing in the second network hydrogel (third row). Figures are on the same scale (all scale bars = 10 mm).

As seen in the Figure 2, the large mesh experienced the highest degree of swelling due to the lower density of fibers. Since the gel could occupy more space between fiber-concentrated areas, it had more leverage to “push” these regions apart during swelling. The smallest mesh size, however, didn’t allow the gel to fill a significant amount of space between the fiber-concentrated areas. Therefore, the fibers stayed tightly woven and inhibited overall swelling of the composite hydrogel.

The meshes were evaluated using SEM before (Figure 3) and after (Figure 4) gel incorporation.

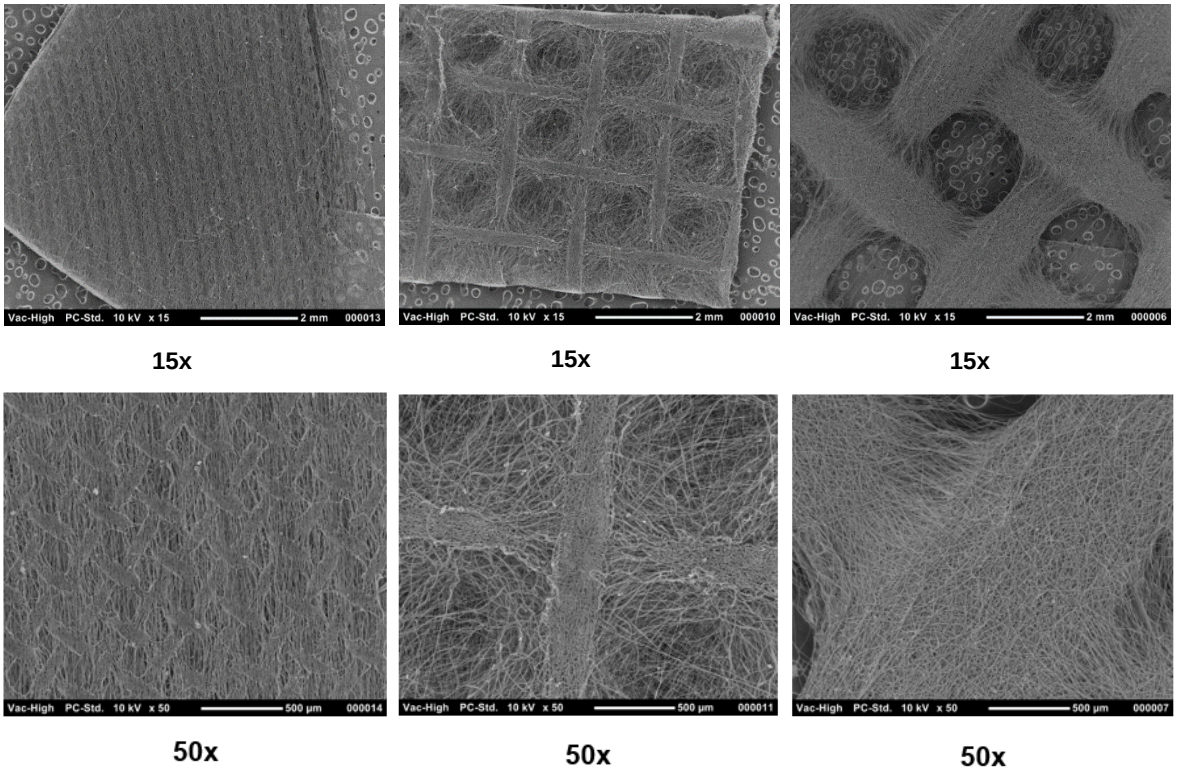

Figure 3. SEM images of meshes before and after incorporation of gel. From right to left: small, medium, and large mesh at 15 followed by 50 magnification.

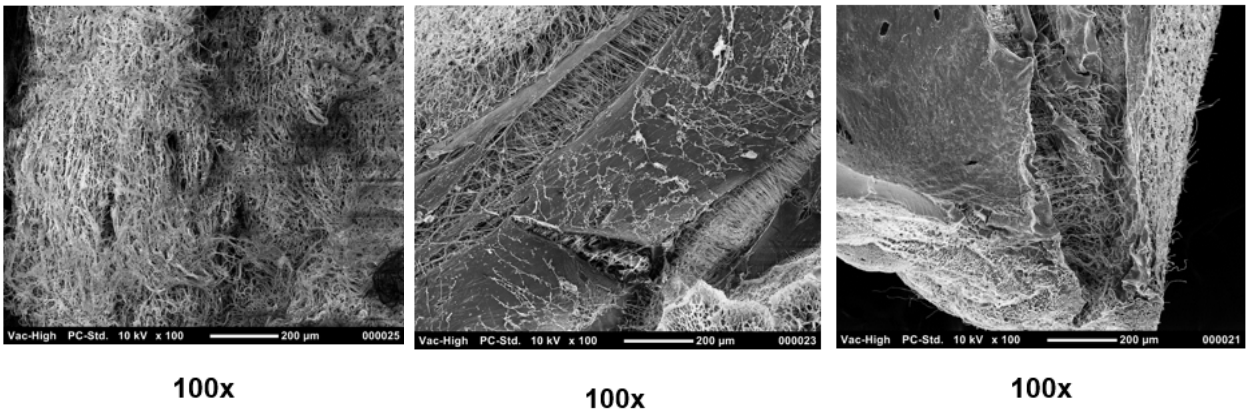

Figure 4. SEM images of meshes after incorporation of gel. From right to left: small, medium, and large mesh.

In the figures taken before gel incorporation, all meshes formed fibers that generally took on the woven structure of the wire mesh collectors. However, the medium and large meshes formed randomly aligned fibers that bridged the gaps in the metal mesh and broke from the overall mesh pattern. The small mesh didn't allow this randomness to occur- the gaps in the mesh were simply too small. The charge of the metal mesh pulled the fibers tight across these "squares," creating diagonally aligned fibers to bridge the gaps while keeping a tightly woven pattern throughout the sample. (Figure 3) This consistent alignment could improve the mechanical properties over the traditional, randomly aligned fiber mats while reducing the density of the fiber mesh to incorporate into a hydrogel matrix. In the SEM images after gel incorporation (Figure 4), the fibers could not be seen on the surface since they were well-integrated within the gel. Thus, to view the fiber-gel integration the samples were fractured to examine the cross-section of the finished composite gels. These images showed thorough incorporation between the two components in every size. Due to challenges with sample preparation of hydrogel specimens for SEM imaging, such as artefactual cracking and pore formation due to freeze drying, the effects of fiber incorporation on hydrogel morphology were difficult to determine.

Varying PCL Fiber Mesh Size

To evaluate mechanical properties, the mesh-gels were tested to fracture in tension. The tensile elastic modulus and strength were recorded and compared to that of the 10% AAm "plain gel," i.e. no fiber mesh. (Figure 5)

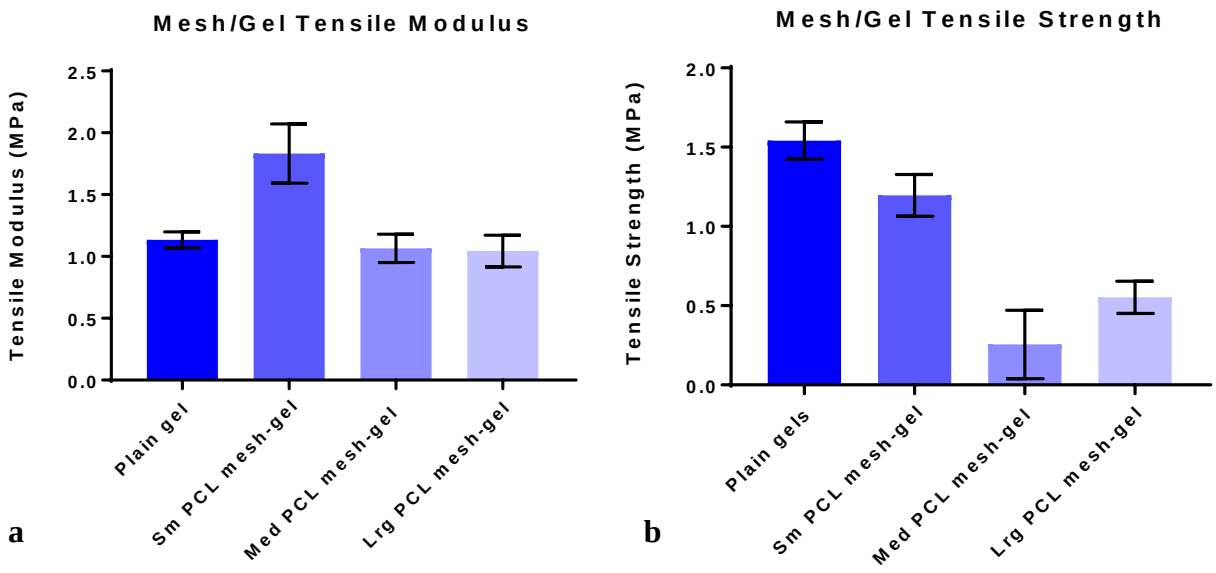

Figure 5. a) Tensile modulus compared between mesh sizes in gels and control plain gel b) Tensile strength compared between mesh sizes in gels and control plain gel

In modulus (Figure 5a), the small mesh-gel performed better than both the control plain gel and the two larger sized mesh-gels. The small mesh-gel had an average tensile modulus of 1.8 MPa, whereas the plain gel had a tensile modulus of 1.1 MPa. This significant improvement of 38.1% is due to the high density of the incorporated fiber mesh providing a greater resistance to deformation. However, in strength (Figure 5b), there was a slight decrease of 22.4% from the small mesh gel to the control plain gel, with the plain gels having an average strength of 1.5 MPa and the small mesh gel having an average of 1.12 MPa. This drop in strength was imparted by the increased brittleness observed in the small mesh-gel. When comparing between mesh sizes, the small mesh-gels outperformed the medium and large mesh-gels in both strength and

modulus. This is potentially due to the more loosely arranged fibers present in the medium and large mesh-gels allowing for discrete gel regions in between the woven fibers to form, compromising the overall structure. Alternatively, the small mesh size provided the organization the fibers needed without compromising the structural integrity of the hydrogel network. Due to the enhancement in properties seen with the smallest mesh size, this composition was used for the remainder of the investigations.

Incorporating NIPAAM into PCL Fiber Meshes

The final method performed in hopes of improving overall integration between the fibers and gel was to add NIPAAM monomer into the PCL solution used to spin the fibers. The prediction was that the hydrophilic properties of NIPAAM would attract more water into the fibers and allow more interaction with the hydrogel precursor solution.

In order to evaluate the effect that NIPAAm had on the fibers prior to gel incorporation, the small fiber mats (with and without NIPAAm incorporation, no gel) were tested in tension. (Figure 6)

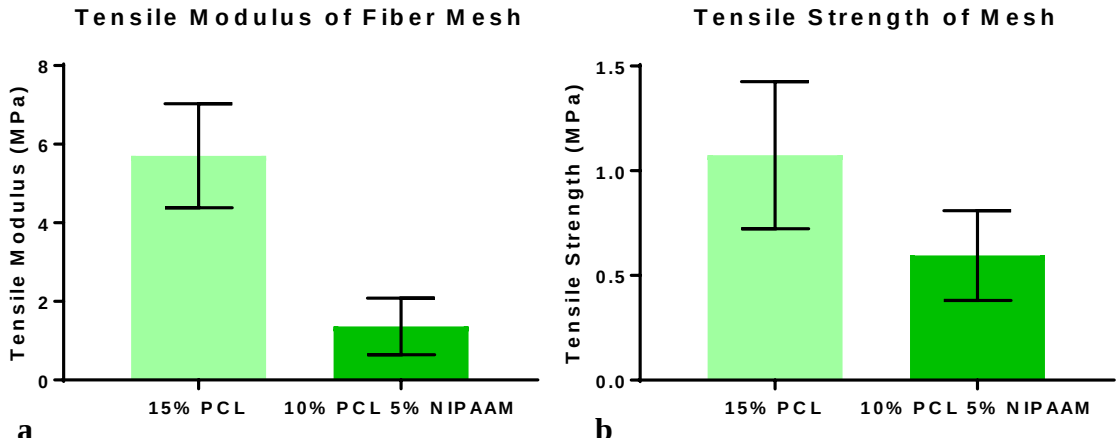

Figure 6. a) Tensile modulus compared between PCL mesh and PCL/NIPAAm mesh. b) Tensile strength compared between PCL mesh and PCL/NIPAAm mesh

The plain PCL meshes had a higher modulus (Figure 6a) and strength (Figure 6b) than the meshes with NIPAAM integrated into them. When making the PCL/NIPAAM fiber solution, the NIPAAM crystals appeared to dissolve, but after the meshes were taken off of the electrospinner and the ethanol and chloroform evaporated, the NIPAAM could have recrystallized in the mesh, interrupting connections between PCL fibers. This compromised the overall structure of the mesh, significantly hindering the tested mechanical properties compared to the plain PCL mesh. Both types of meshes were combined within gels and tested in tension. (Figure 7)

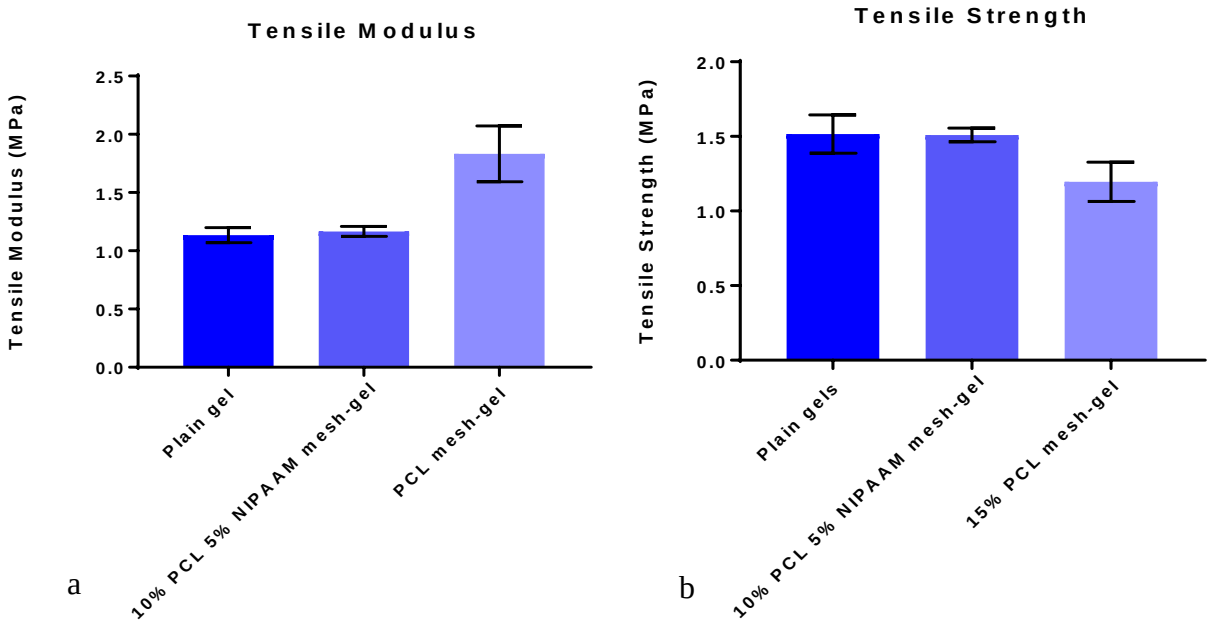

Figure 7. a) Tensile modulus compared between gels containing PCL mesh and PCL/NIPAAm mesh. b) Tensile strength compared between gels containing PCL mesh and PCL/NIPAAm mesh. The mesh-gels with NIPAAM incorporated into gel solution showed similar properties to that of the plain gels, whereas the PCL mesh-gels only showed improvement in tensile modulus.

The meshes containing NIPAAM, when combined within hydrogels, showed similarity to the plain gel control in both modulus and strength, whereas the meshes containing only PCL showed improved modulus but lower strength compared to the control (Figures 7a and 7b). If the

NIPAAM crystalized in the PCL/NIPAAM meshes, it may have dissolved immediately upon exposure to the first network gel solution. Even though this successfully allowed for the PCL/NIPAAM mesh to integrate better with the gel solution, the dissolution of the NIPAAM could have left inhomogeneities in the fiber mesh. Therefore, these PCL/NIPAAm mesh-gels showed no significant improvement over either individual components (plain gel or PCL/NIPAAm fiber mat). On the other hand, the PCL-only meshes exhibited considerably higher modulus (~6 MPa, Figure 6a) and would not undergo this dissolution during gel incorporation, thus producing a substantial reinforcement. Therefore, the PCL mesh-gel combined the desired high modulus of the fiber mat with the high strength of the plain hydrogel to achieve a superior composite mesh-gels with both high stiffness and strength.

Notch Test

To demonstrate the injury resistant properties that the fibers imparted on the gel, a "notch test" was performed. As shown below in Figures 8 and 9, the small PCL mesh-gels showed great improvement at resisting tearing after an initial cut had been made into the gel.

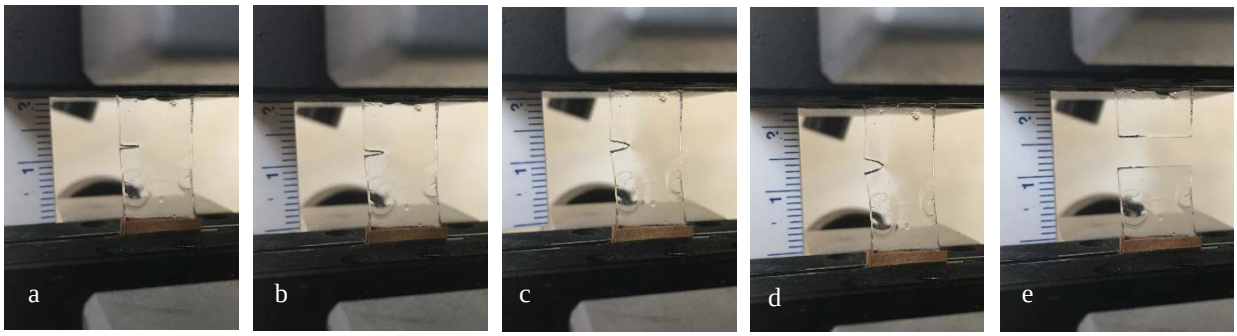

Figure 8. Plain gel notch testing. Notch only grew to approximately 3 mm wide prior to fracture.

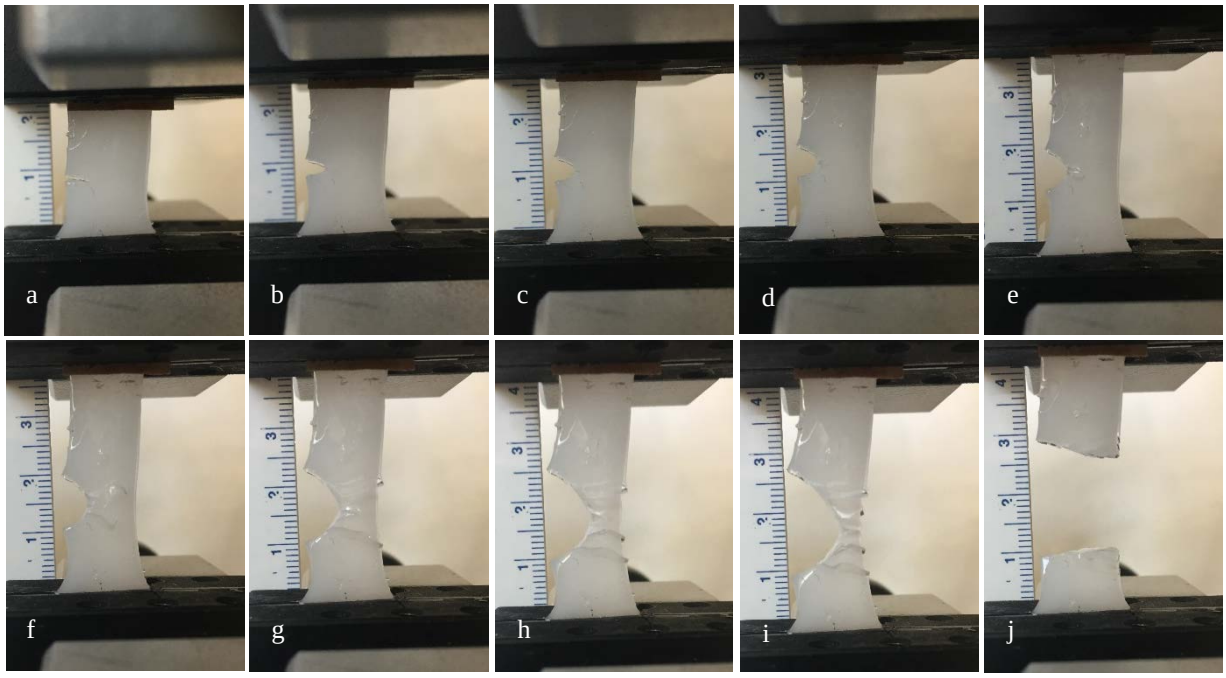

Figure 9. Mesh-gel notch testing on small PCL mesh-gel. Notch grew to approximately 2.5 mm wide before fracture.

The gels with no fiber incorporation broke quickly in one clean motion, with little resistance to tear as the tensile force was applied (Figure 8). The notch in the mesh-gels grew slowly, with smaller tears in the gel surrounding the fibers. The mesh-gels stretched in a ragged manner until only a tiny sliver was holding the mesh-gel together before complete fracture, sustaining a much higher percent strain. Figure 9 illustrates the fibers at work maintaining the integrity of the hydrogel slab under tension. In Figure 9e-i, the gel cracks around the fibers, but the fibers continue to stretch in response to the tensile force.

As shown in Figure 10, the mesh-gels showed a substantial improvement in percent strain at fracture. The mesh gels reached an average of 76.7% strain at break, whereas the plain gels only attained an average of 21.8% stain at break. This is a 71.6% improvement, showing that the fibers can successfully hold the structure of a damaged gel together under an applied tensile force. The PCL mesh-gels were able to stretch almost four times the length compared to the control gels. In

a clinical situation, these mesh-gels could provide a reliable synthetic grafting material to patients and clinicians that can withstand minor mechanical injuries and still perform well in the body.

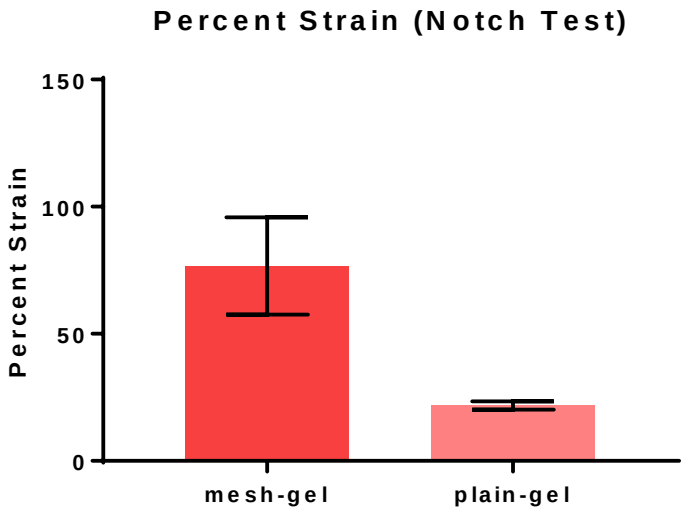

Figure 10. The percent strain of the gels at complete break.

To further demonstrate the robustness of these mesh-gels, toughness during the notch test was also analyzed by calculating the area underneath the stress-strain curve, as shown in Figure 11. The mesh-gels had an average toughness of 0.26 MJ m^{-3}, and the plain gels had an average toughness of 0.02 MJ m^{-3}. This is a 91.2% increase. Again, this shows the significant improvement that incorporating fibers into gels made in increasing resistance to injury in the body. The high durability of these hydrogels would provide a more reliable synthetic grafting material that maintains the desirable hydration and lubricity of traditional hydrogels.

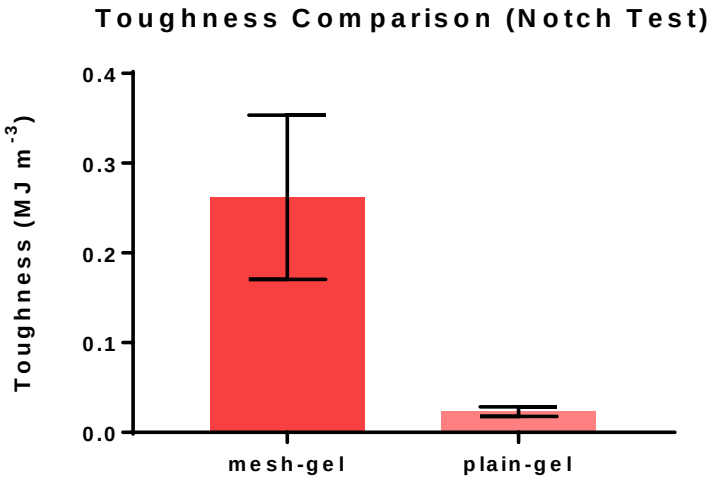

Figure 11. Toughness during notch test of mesh-gels vs plain gels.

Making Gels with Exposed Fibers

To demonstrate that gels could be created with exposed fibers as a possible suturing application, different shaped gels with exposed fibers were fabricated. This was accomplished by only pressing the 1st network solution into areas where gel incorporation was desired. In Figure 12, these unique gels are shown after 1st network incorporation and after 2nd network incorporation. Swelling of the 2nd network presented an issue, as the fibers with gel incorporation could warp the surrounding "dry" fibers. However, with a more sophisticated mechanism for clamping the gels in place after curing, this issue could be addressed. These preliminary results show great promise in the fabrication of mesh-gels with exposed fibers to directly suture to tissues as grafts or other biomedical devices.

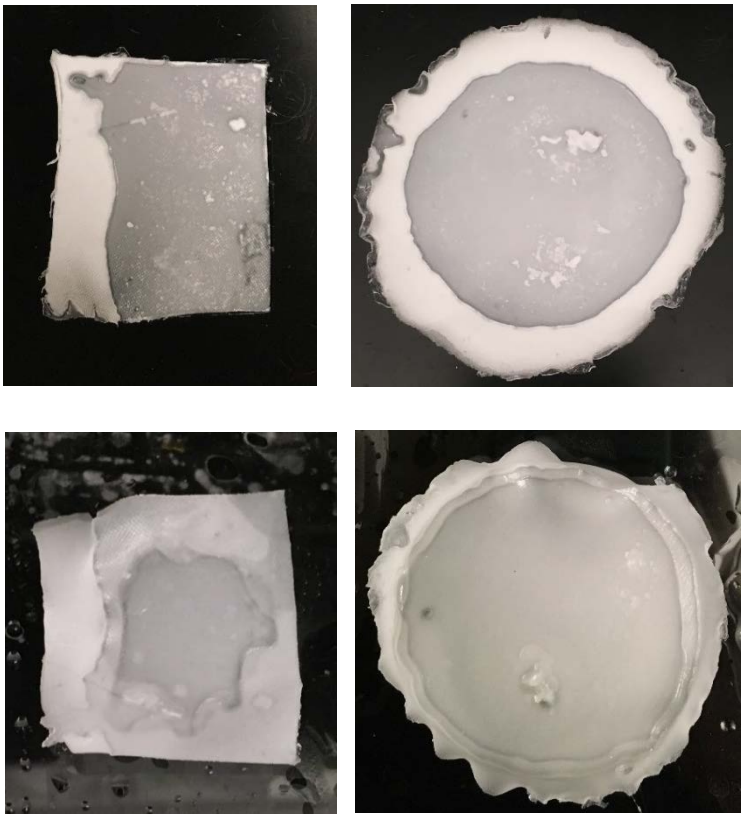

Figure 12. Gels with exposed fibers after 1st network incorporation (first row) and after 2nd network incorporation (second row).

CHAPTER IV
CONCLUSION

In summary, this work led to a unique fiber reinforced DN hydrogel with the impressive combined properties of ultra-high strength (~1.2 MPa), improved stiffness (~1.8 MPa) and enhanced tear resistance (>4x plain gel). This was achieved by incorporating patterned electrospun PCL fiber mats within a previously established ultra-strong DN hydrogel. Ultimately, the smallest size mesh (with holes ~0.10 mm) was determined to be the most appropriate for obtaining the optimal mechanical properties. To further promote integration of the fiber mesh with the gel solution, NIPAAm was added to the PCL fiber solution to increase hydrophilicity of the fibers. Although the PCL/NIPAAm meshes did encourage interaction of the fiber mesh and gel solution, the mechanical properties were diminished. Research on other methods to enhance fiber-gel integration is necessary to consistently obtain high quality mesh-gels. However, small PCL mesh-gels made through mechanically forcing the solution into the fiber mesh were adequate for preliminary notch testing. As expected, the PCL mesh-gels performed extremely well under notch testing conditions, with large increases in both toughness and percent strain compared to the control plain gels. The remarkable mechanical properties along with the improved resistance to injury achieved by the PCL mesh-gels provides great potential as candidates for synthetic cartilage grafts.

Alternative Applications

Overall, the incorporation of electrospun fibers maintained the high strength of the DN hydrogels while enhancing both modulus and tear resistance. This provides an avenue towards applications that require suturing of hydrogels. Gels that can resist injury are better suited to

withstand the mechanical demands of connecting to another device. Due to their hydrophilicity, it is often difficult to adhere hydrogels to the surface of devices made from metal or other types of polymers. Therefore, a mesh-gel with exposed fibers provides an opportunity for anchoring gels to such devices. With an interpenetrating network of fibers within the hydrogels, exposed fibers could be easily positioned to hold the gels in place through wrapping or suturing. Additionally, these fibers could provide a method to suture hydrogels to tissue, reducing complications which arise in grafting procedures from implant loosening.

Future Directions

To further investigate these mesh-gels, a better protocol could be developed to clamp the gel in place as they swell after curing to prevent the gel from folding and twisting the fibers out of their original planar shape. Moreover, the fibers could be made into more novel 3D shapes (i.e. cylinders) and then incorporated into hydrogels for applications as vascular grafts. Advanced methods of testing could be employed, such as evaluating suture strength, examining friction against cartilage or measuring wear over time during simulative knee motion.

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